REMARKS

Status of Claims

Claims 3-5, 9-12, 14-15, 18, and 21 are pending in this application. Claims 7 and 20 have been canceled in this amendment. Claims 3, 9-10, and 14-15 have been amended to more particularly point out and distinctly claim that which the applicants regard as their invention. Support for the claim amendments can be found, *inter alia*, in the original claims, and in paragraphs [0007], [0081], and [0144]. Claim 3, in particular, has been amended to clarify the language of the original claim. Step iii) of the method was intended to involve the selection of the bound cells that express CD133. While the applicants believe this selection step was adequately described in the original claim, in order to clarify the language, the claim has been amended herein. No new matter has been added by these amendments.

Claim Rejections under 35 U.S.C. §112

The rejection of claims 9-10, and 14 under 35 U.S.C. §112, second paragraph, as being indefinite is believed overcome by the amendments to the claims. Specifically, the claims have been amended to ensure that there is proper antecedent basis for each of the terms in the claims.

Claim Rejections under 35 U.S.C. §103

The rejection of claims 3-5, 7, 9-12, 14-15, 18, and 20-21 under 35 U.S.C. 103(a) as being obvious over Collins et al. (2001), J. Cell Science, Vol. 114, 3865-3872 (hereinafter "Collins (2001)"), in view of Mangano (US 2007/0134794) is respectfully traversed.

The presently claimed invention is directed to a method of isolating prostate cancer stem cells which express the CD133 antigen, CD44 antigen, and high levels of $\alpha_2\beta_1$ integrin. The claimed method starts with a population of prostate cancer stem cells, and proceeds by binding the prostate cancer stem cells to a collagen matrix, selecting the bound cells that express the CD133 antigen, and isolating bound cells that express CD133 antigen, CD44 antigen, and $\alpha_2\beta_1$.

Collins (2001) teaches a method for isolating a population of prostate stem cells derived from non-cancerous prostate cells, wherein the selection is based on the expression of

CD44. Collins (2001) is silent on the use of CD133 antigen as a marker for prostate cancer stem cells.

Mangano is cited for its teaching of methods of selecting for and enriching stem cells. While Mangano describes the isolation of prostate stem cells from sources such as solid tumors or metastatic tissue, Mangano does not disclose a method of isolating prostate cancer stem cells based on the CD133 marker.

The Office alleges that while the references do not disclose or suggest a method of isolating prostate cancer stem cells based on the expression of the CD133 antigen, a method that selects prostate stem cells based on CD44 expression and collagen adherence results in a cell population that inherently expresses CD133. This allegation of inherency is not correct as evidenced by Collins et al. (2005), Cancer Res., 65(23): 10946-51 (of record), hereinafter referred to as "Collins (2005)". Collins (2005) demonstrates that it is possible to have cells that express CD44 but do not express CD133. In view of the evidence provided in the Collins (2005) reference, it cannot be said that expression of CD44 inherently means expression of CD133 because expression of CD44 and CD133 are not directly linked to each other. Simply put, Collins (2005) proves that not all cells that express CD44 express CD133. Therefore, it is incorrect to state that CD133 selection is inherent in the method of Collins (2001), which selects only for CD44.

As further evidence that a method of isolating prostate stem cells comprising selecting cells for CD44+ expression and for rapid adherence to collagen, as taught by Collins (2001) would not inherently result in the isolation of prostate stem cells that express both CD44 and CD133, as alleged by the Office, the applicants point to the Richardson et al. article cited by the Office, ((2004) J. Cell Sci., Vol. 117, 3539-3545). Like the Collins (2005) article, the Richardson et al. article is also not prior art over the present application because it was published after the effective filing date of the present application. In Collins (2005) the authors teach that the CD44+/$\alpha_2\beta_1^{hi}$/CD133+ cells were isolated using the methods previously described by Richardson et al. The method taught in Richardson et al. starts with cells that have been selected for expression of CD44 and fractionated on the basis of adhesion to type I collagen, as described in Collins (2001) ***and further selects the cells for CD133 expression*** (see page 3540, column 1, second full paragraph). In other words, the starting cells in Richardson et al. are all CD44+, but are not necessarily CD133+, and a second selection step

is necessary to isolate cells that are CD133+. If the cells resulting from the method taught by Collins (2001) already contained an isolated population of cells that express both CD44 and CD133, as the Office alleges, then the further purification taught in Richardson et al. would be completely unnecessary. Instead, Richardson et al. teaches that after the second purification step selecting for CD133 expression, there were two fractions, one that was CD133-positive, and one that was CD133-negative (see page 3540, column 1, second paragraph). Thus, the article cited by the Office further supports that isolation based on CD44 expression and adherence to collagen does not result in an isolated population of prostate stem cells that express both CD44 and CD133.

Furthermore, the presently claimed method includes a step that selects for prostate cancer stem cells that express the CD133 antigen. As the Office previously concedes, neither of the cited references (Collins (2001) nor Mangano) teach that prostate cancer stem cells express the CD133 antigen (see page 9 of the Office Action dated December 7, 2010). Because neither of the cited references recognize the significance of the expression of CD133 in either prostate stem cells or prostate *cancer* stem cells, one of skill in the art would have no motivation to select prostate stem cells based on the expression of this antigen. Additionally, as described above, the expression of CD133 in prostate stem cells is not inherent to all prostate stem cells that express CD44. Thus, one of skill in the art would not have had a reasonable expectation of successfully isolating a population of prostate cancer stem cells that express both CD133 and CD44 by isolating the cells on the basis of only CD44 expression and adherence to collagen.

Claims 15 and 18, directed to a cell culture of prostate cancer stem cells, have amended to recite particular features of the cell population that are not disclosed in the cited references. In particular, the claims have been amended to recite that prostate cancer stem cells that express CD133 antigen, CD44 antigen, and $\alpha_2\beta_1$ integrin have high *in vitro* proliferative potential, have higher colony forming efficiency than $\alpha_2\beta_1$ integrin^{low} CD133$^-$ prostate cells, and that the cells can form cancerous prostatic-like acini in an immune-compromised non-human animal model. These unique features were not disclosed or suggested in either of the cited references. Additionally, in regard to prostate cancer stem cells that express CD133 antigen, CD44 antigen, $\alpha_2\beta_1$ integrin, and human epithelial antigen (claim 18), this population of cells was also not disclosed or suggested in the prior art. As

described above, a population of cells that have been isolated based on the expression of CD44 do not also inherently express CD133, and thus, the population of cells in the present claims that must express both CD44 and CD133 is not disclosed in either of the references. And, because the method of isolating these cells in the present application is different than the method of isolation taught in Collins (2001), it cannot be said that the presence of human epithelial antigen is inherent in both populations. Therefore, for at least these reasons, claims 15 and 18 are also patentable over the cited references.

For all of the above reasons, the combination of Collins (2001) and Mangano do not render the presently claimed invention obvious, and reconsideration and withdrawal of the rejection are respectfully requested.

Conclusion

The application is respectfully submitted to be in condition for allowance, and prompt, favorable action thereon is earnestly solicited.

If there are any questions regarding this reply or the application in general, a telephone call to the undersigned at (202) 624-2845 would be appreciated since this should expedite the examination of the application.

If necessary to effect a timely response, this paper should be considered as a petition for an Extension of Time sufficient to effect a timely response, and please charge any deficiency in fees or credit any overpayments to Deposit Account No. 05-1323, Docket No. 100846.59603US.

Respectfully submitted,

November 29, 2011

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